SOME OBSERVATIONS ON THE GROWTH OF *RHIZOCTONIA CROCORUM* (PERS.) DC. IN PURE CULTURE

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SOME OBSERVATIONS ON THE GROWTH OF *RHIZOCTONIA CROCORUM* (PERS.) DC. IN PURE CULTURE

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(With Plate X and 3 Text-figures.)

Name of the fungus.

The fungus with which these notes are concerned is that which causes the well-known "Violet Root Rot" of various crops, such as lucerne, potatoes, carrots, etc., and which has hitherto been generally known in this country as *Rhizoctonia violacea* Tul. The specific name *violacea* was given by the Tulasne brothers (1) in 1851 when they reduced to a single species the older forms known as *R. Crocorum* and *R. Medicaginis*. This proceeding is however contrary to the Rules of Nomenclature now accepted. Assuming, as do the majority of workers, that the Tulasne brothers were right in their view that the violet root disease in all the various crops is caused by one and the same species of fungus, even though there may be biologic forms, then the earliest valid name, and not that of the Tulasnes, must be adopted. As pointed out by Duggar (2) the correct combination is *Rhizoctonia Crocorum* (Pers.) DC. The specific name was originally used as *Sclerotium Crocorum* by Persoon (Synopsis, 1801) for the form on *Crocus sativus*. It was transferred to his genus *Rhizoctonia* by De Candolle in 1815, and both this and De Candolle's second species, *R. Medicaginis*, were taken up by Fries in the Systema Mycologicum (1823), which latter is the starting point for Nomenclature according to the International Rules. It is unfortunate that the name is less descriptive than *R. violacea*, and is particularly inappropriate in this country where the Crocus disease is unknown. Pending further information as to the perfect stage, however, the name *R. Crocorum* must stand.

Origin of the present work.

In the winter of 1922–23 a considerable amount of circumstantial evidence was obtained pointing to the possibility that *R. Crocorum* might

have as its perfect stage the fungus *Helicobasidium purpureum*. The authors therefore set out on an attempt to test the theory by pure culture and inoculation experiments. They were fortunate in being able to obtain comparatively abundant material of the *Rhizoctonia* on clover and later also of *Helicobasidium* through the kindness of Mr W. M. Ware of the South-Eastern Agricultural College, Wye. Owing to unexpected complications it has not yet been possible to obtain sufficient evidence for a definite pronouncement, but the authors hope to be able to give some account of this investigation after a more complete set of inoculation experiments has been carried out.

The present notes are the outcome of one side of the work, namely, the successful and continued growth of *R. Crocorum* in pure culture. The only previous records of artificial cultures of this fungus known to the authors are those of Diehl(3) in America and van der Lek(4) in Holland, neither of whom carried out very extensive cultural work with it. The former grew the *Rhizoctonia* chiefly in mixed culture with a species of *Fusarium* and did not obtain good growth in the pure state. Van der Lek succeeded in isolating the fungus pure from sections of the "infection cushions" or "corps miliaires" and obtained good growth on malt agar. He also grew it on cherry and dung extract agars and gives some account of variations in colour according to the medium and the amount of moisture present. We understand that both of these workers allowed their cultures to die out. Faris(5) writing in 1921 stated that "cultural experiments to secure pure cultures of the organism (*R. Crocorum*) are under way" but the authors have seen no account of successful cultures. In a letter to one of the authors Dr G. H. Pethybridge stated that he grew the fungus successfully, although not in pure culture, by keeping an attacked potato tuber in a covered dish with other healthy potatoes. In time the *R. Crocorum* grew luxuriantly over the whole lot and its "corps miliaires" penetrated the skins.

Methods used for isolation in pure culture.

After numerous unsuccessful attempts in the winter of 1922–23 growth of *R. Crocorum* was obtained in pure culture on malt agar and on a meat and malt extract gelatine acidified with citric acid[1] by adopting the method of van der Lek(4), viz. planting moderately thick sections of "infection cushions" of the fungus, cut with a razor under aseptic conditions, on previously poured plates of the media. One

[1] This gelatine was made up with tap water 1000 c.c., malt extract 20 gm., Lemco meat extract 10 gm., citric acid 4 gm., gelatine 120 gm., and cleared with white of egg.

of the sections showing promising growth was sacrificed for high power microscopic examination and the developing hyphae were seen to be growing out from the centre of the micro-sclerotium as described by van der Lek. The material used was that on Red Clover, mentioned above, supplied by Mr W. M. Ware (cf. (6))[1]. Van der Lek (*loc. cit.*) gives no details of his method of surface sterilisation of his plant material. The authors obtained best results from the use of a 50 per cent. alcoholic 1/1000 mercuric chloride solution with technique similar to that adopted by bacteriologists (cf. Manns(7)). Portions of the clover root bearing numerous infection cushions or micro-sclerotia were put into sterile test tubes and well covered with the mercuric chloride solution. After a given interval the disinfectant was poured off and the material washed at least three times with separate portions of sterile distilled water. Each time the liquid was shaken to all parts of the tube and on changing the liquid the old cotton wool plug was replaced by the dry sterile plug from the new sterile water tube. The material remained in the original tube throughout the operation. It was found that about one minute in the mercuric chloride solution was ample to kill all really surface contaminations: longer exposure usually resulted in absolutely sterile sections—killing also the micro-sclerotia of the *Rhizoctonia*. After surface sterilisation the material was transferred to a sterile petri dish, portions cut off from the root tangentially $\frac{1}{2}$–1 mm. thick and transverse sections of the micro-sclerotia cut in sterile pith. The razor was flamed after dipping in alcohol before use. The difficulty which has been experienced by previous workers (cf. Duggar, *loc. cit.*) in obtaining growth of *R. Crocorum* on artificial media has been occasioned in part by the apparently low degree of vitality possessed by such minute portions of mycelium as can be transferred without accompanying contaminations from the advancing edge of growth of the fungus, *e.g.* at the collar of infected plants. In the opinion of the authors the main difficulty has been due *not* to the fungus being on the borders of obligate parasitism but to the difficulty of seeing and isolating the hyphae, which grow out slowly from viable portions of the fungus, *e.g.* the micro-sclerotia, before they have been swamped by other more quickly developing fungi and bacteria. In the field it is rare to find many plants attacked by the parasite and the choice of material is rather limited. Further, from the fact of the *Rhizoctonia* occurring mainly on the underground parts of plants, it is

[1] A further case in this country of a very slight attack of *R. Crocorum* on Red Clover has since been noted by us in Hampshire. *R. Crocorum* has also occurred in one of our experimental pots on another species of clover (*Trifolium hybridum*).

always associated with very numerous rapidly growing saprophytes and in many cases before the attacked parts are dug up these saprophytes have already obtained a hold on the tissues affected by the *Rhizoctonia*. In cases where the razor sections of the micro-sclerotia were "planted" free from contaminations and where the surface sterilisation of the roots had not been too severe the *Rhizoctonia* grew out slowly but steadily on to the artificial medium[1]. When suitable conditions were found for the growth of the fungus it grew at least as freely in culture as some other parasitic fungi whose occurrence on the aerial parts of plants comparatively free from contaminations, combined with the production of abundant conidia, has led to their being isolated in pure culture without difficulty. The very slow growth of certain parasites, such as species of *Pseudopeziza*, in artificial culture has been noted particularly by Klebahn (8).

Confirmation of the identity of the organism isolated.

The mycelium which grows out from the infection cushion or micro-sclerotium is at first colourless, and the hyphae very slender, but very soon the characteristic features of the branching of *Rhizoctonia* can be detected. After the lapse of two to three weeks at laboratory temperature the colony shows the typical violet colour and the older hyphae are now seen to be assuming the size and colour characteristic for *R. Crocorum*. The centre of the colony gradually becomes deep violet or brownish, according to the medium, and at the same time the substratum becomes permeated with the densely matted branching hyphae, so that a tough, compact mass is formed which is very difficult to cut with the usual platinum needles used for making sub-cultures.

The hyphae of *R. Crocorum* obtained in culture do not differ essentially from those of the fungus in nature, as described and figured by Duggar (*loc. cit.* p. 415). The normal well-grown hyphae are usually about 4–6μ in diameter. The younger hyphae are pale, especially towards the tips, with rather distant transverse septa. At the apices the hyphae contain dense protoplasm, but further back they become vacuolate, while older hyphae become thick-walled, rigid and deeply coloured, and appear to contain little or no protoplasm. Branches arise usually singly, more or less at right angles to the main hypha, and near to the

[1] A contributing factor to the slow growth obtained at first was that it was necessary to use a fairly thin layer of medium, with consequent drying out, in order to be able to watch the growth with the low power of the microscope through the bottom of the petri dish.

cross walls. Each branch is cut off by a septum close to the main hypha, and frequently appears to be slightly narrower near the point of origin. Duggar's observation that at the point of union the diameter of the branch is uniform with that of the main hypha applies more to the older, coloured hyphae. The experience of the writers has been that the younger hyphae, both in nature and in culture, usually show this slight narrowing where the branches join the parent hyphae. The same observation has been made by Faris(5). Occasional fusions between adjoining hyphae take place, but no clamp connections have ever been observed.

In order to confirm the results of microscopic examination and to show more definitely that the fungus isolated was really *R. Crocorum* inoculation experiments were carried out. As a preliminary test, on 29th July, 1923, portions of mycelium from a pure culture were placed in contact with young carrots and potatoes, the surface of which had been carefully washed, treated with dilute formalin and again washed. Two potatoes and three young carrots thus inoculated were kept moist in a large covered glass dish. In the case of the potatoes there was a very slow growth of scattered hyphae over the surface of the tubers but no further development. With the carrots, however, better results were obtained. On 1st September one of the carrots showed a distinct patch about 1 cm. in diameter of typical *R. Crocorum* attack with minute infection cushions, while a second root showed also incipient attack and a few infection cushions. In both cases the affected parts became gradually much larger until finally both carrots were completely destroyed. Secondary organisms no doubt contributed to the rot.

A further inoculation experiment was carried out with young growing carrots. Some seedling carrots had been carefully transplanted into two large pots of soil which had been sterilised by steam by the usual method for gardening operations. By 30th July these were well established and sufficiently large for inoculation. In one pot fairly big pieces of growth cut from pure culture were inserted in the soil close to the crown of each plant. The second pot was left untouched as a control. By 17th September one carrot in the inoculated pot was seen to be dying off and when pulled up was found to be covered with the typical infection cushions of *R. Crocorum*. By 18th October a second carrot had completely rotted and the skin remaining was found to be full of infection cushions. Later a third carrot died and was found to be attacked in the same way, while another showed slight infection. In the control pot no sign of *Rhizoctonia* ever occurred. From one of the infected carrots the parasite was re-isolated by the same method as used originally. The

growth in artificial culture was found to be exactly similar to that obtained from the clover material.

A similar inoculation test was carried out with Red Clover. Seeds of Red Clover had been sown in large pots in sterilised soil in July 1923 and on 3rd September the young plants in one pot were inoculated by placing portions of a pure culture in the soil close up against the upper part of the tap-root of each plant. On 2nd November further mycelium from a pure culture was introduced. A pot was left uninoculated as a control. The pots were kept in a small unheated glasshouse, and as from the time of the first inoculation the weather was never warm results were doubtless obtained much more slowly than they would have been had it been possible to inoculate earlier in the season. On 11th April 1924 all the clover plants were pulled up and examined. Of those in the inoculated pot five out of eight showed typical attack by "Violet Root Rot." Infection cushions occurred both on the tap-roots and on some lateral roots, while in one case the tap-root had completely rotted away leaving only a short stump. The clovers in the control pot were found to be all quite healthy.

There seems to be no doubt therefore that the organism isolated and grown in pure culture is really *R. Crocorum*.

The successful inoculation of carrots with a pure culture obtained from clover plants is of some significance in connection with the view expressed by Eriksson (9) that the violet root rot of the carrot and of lucerne (a legume) are two entirely different things. Eriksson considered that the sterile fungus called *R. violacea* (*R. Crocorum*) might include several biologic forms or even species. He based this opinion in the first place on the results of some cultural experiments. Using infected soil in which a crop of diseased carrots had been grown, he got only a slight infection on lucerne and none on Red Clover or parsnip; in one experiment made with cross-inoculation in the reverse direction, from lucerne to carrot, he got no result. Similarly a test with forms of the fungus on lucerne and asparagus indicated that these would not pass from one host to the other. From these experiments and from records of the occurrence of the alfalfa (lucerne) disease and of attacks on Red Clover in Germany (the former frequent, the latter rare) he argued even that the lucerne and the clover forms might be distinct.

Seeing that our present knowledge is so slight, however, both as to the requirements of the fungus and the conditions necessary for successful infection of its various hosts, Eriksson's experiments require to be carefully repeated.

Eriksson's further argument that the perfect stage of the carrot fungus is his *Hypochnus violaceus* and that of the alfalfa fungus *Leptosphaeria circinans* is generally regarded as being based on insufficient evidence, and need not be considered here.

Growth on various media.

Diehl (3) obtained his best growth of *R. Crocorum* on "radicicola" agar acidified to 5 on the scale given in old editions of the American Standard Methods of Water Analysis. As the authors were unable to have access to an old edition it was impossible to find how much added acetic acid was represented by the number 5, but from the description given by Diehl it would appear that very much better growth was obtained on the media generally used in the present work than that given by the acidified "radicicola" agar. Van der Lek obtained good growth on malt agar.

The authors have found that media made up with malt or meat extract or preferably both, and also prune agar, allow of best growth, and these have been chiefly used. When first isolated, however, transfers were made to a great variety of media with the aim of finding as early as possible which would be the most suitable, so that there should be no risk of the organism dying out when once obtained in pure culture. Pure growth was sparse at the time so that only a few tubes of each were inoculated. Further, in some cases the agars available had been prepared for some time, and it has been noted from later work that there may be appreciable differences, particularly in the amount of aerial growth, when freshly tubed agars are used. The results are, however, given here in brief in order to show on how wide a range of sterilised artificial material growth of *R. Crocorum* will take place when it is not overwhelmed by the presence of other more rapidly growing organisms.

As an illustration of the way in which the development of aerial mycelium, and hence the general appearance of the culture, depends on the amount of condensation moisture present one case may be given where four malt-meat extract agar plates were poured at the same time, and inoculated with as nearly as possible equal and very minute fragments of mycelium from the edge of a recent culture. The plates were kept in an incubator at 22–23° C. It happened that two of these plates included a considerable amount of water of condensation while the other two remained fairly clear and dry. After about four weeks the differences in growth were striking. In the two plates with much moisture

there was good development and a soft growth of pale lilac-brown aerial hyphae similar to that occurring in fresh tubes. The plate with most free moisture gave a colony 25 mm. in diameter while the one with slightly less moisture measured 13 mm. The other two plates, which had remained clear and dry, showed very much less growth—less than 10 mm. in diameter—and the mycelium was practically entirely submerged, the colony therefore appearing dark purple in colour with no paler aerial growth. On the other hand, while endeavouring to obtain conditions under which the fungus would give regular and equal growth on duplicate plates in artificial culture it was grown on malt extract and meat-malt extract agars made up with 1, 1½, 2, 3 and 5 per cent. of agar, and the varying percentage of contained agar *per se* did not appear to have any appreciable effect on the growth of the organism either at 19° C. or at 30° C.

The microscopic characters of the hyphae may vary somewhat according to the amount of moisture available. In vigorous cultures on freshly-made agars the marginal hyphae grow out fairly straight, with rather infrequent branches. On plates which are drying out, on the other hand, the hyphae become more flexuous and branching is more frequent. Finally in the compact tissue of cultures some weeks old irregular swollen cells may be found resembling those figured by Duggar (*loc. cit.* p. 418, fig. 3) as covering the surface of large sclerotia.

Bodies resembling sclerotia in form occasionally occur, especially as cultures dry out, but the structure of these is more compact and consists of more regular hyphae than Duggar describes for the large sclerotia occurring in nature. The surface of such bodies is, however, often covered like the sclerotia with a layer of short, irregularly branched and thickened hyphae. These sclerotia-like bodies found in cultures appear to be extreme developments of the compact, heaped up growth which occurs at low temperatures rather than special structures such as the sclerotia of *Botrytis*, etc.

Duggar notes that the violaceous pigment of the young hyphae as found in the field is soluble in acidulated water. The authors found that when the growth was reasonably well aerated, as a general rule, abundant pigment was formed and this readily diffused out into the acid gels or liquid cultures. In the latter where the submerged mycelium could be observed it usually appeared colourless, however deeply coloured the medium or the surface growth might become. In this connection it is interesting to note the statement of Faris that "very little pigment is produced after the mycelium enters the host." The absence of colour

within the host tissues has also been noted by previous workers. The colour in liquid cultures was found to be unaffected by heat and to be insoluble in such solvents as ether, chloroform, etc.

With many of the liquid and some of the agar cultures rolled filter paper, and pieces of stick or glass rod, were placed in the flasks before sterilisation with a view to giving the organism an opportunity of climbing up out of the medium to produce fructifications in the free air. Mycelium grew freely upwards on these supports but in no case has any sign of fructification been observed either on such growth or in ordinary cultures.

Malt agar. Growth fairly good, more brownish than purple. The mycelium piles up into compact growth several millimetres above the original level of the agar and in fresh tube cultures some loose aerial mycelium is produced. In petri dishes, where less moisture is present, the superficial growth is more even, compact and granular in appearance. In old colonies the surface growth becomes light brown, sometimes almost white. Deep brownish colour is developed in the medium, but not extending far beyond the edge of the colony. In a flask culture, after $8\frac{1}{2}$ months at laboratory temperature the agar has turned a deep brown colour and the mycelial growth has become piled up into warted excrescences nearly 2 cm. in diameter. The surface of these excrescences changed in appearance from time to time, sometimes appearing deep brown, almost black, in colour and occasionally extruding drops of brownish liquid; at other times it became lighter in colour with a more pruinose appearance, and occasionally a violaceous tint. Extending irregularly over part of the surface is loose rusty brown coloured mycelium. Similar brown mycelium extends up the glass walls in places to a height of 2–3 cm.

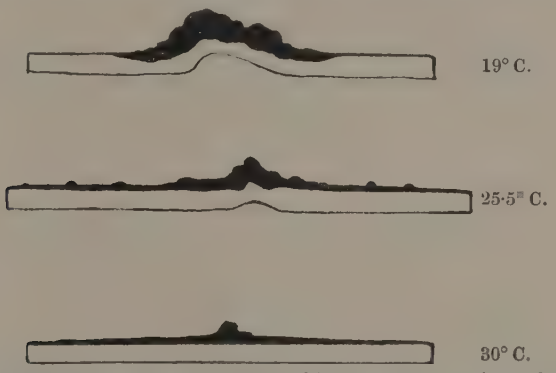
Malt and meat extract agar. Growth good—definitely the best observed. With freshly sterilised moist agar a fair amount of loose aerial mycelium develops. The colour is variable from browns to the typical violet colour of the organism. A deep purplish or brownish colour is developed in the medium. On fairly thick layers of agar in petri dishes growth appears fairly regular and dense over the whole colony at 30° C. but the violet tinge to the brown colour is shown best at 25° C. where the growth aggregates into small rounded sclerotia-like masses 2–3 mm. in diameter. At 19° C. the growth tends to pile up at the middle of the colony with the excretion of numerous drops of dark brown liquid and is very noticeably less dense than at higher temperatures over the wide outer zone of the colony. Plate X shows the appearance of typical colonies grown at these temperatures for five weeks. "Staling" has begun—particularly at 25·5° C.—as indicated by the irregular edge of the colony. In Fig. 1 vertical sections through the middle of each of these plates are shown, in order roughly to compare the total amounts of growth. This however is only approximate, as owing to the very dark colour produced in the medium it is impossible to fix exactly the limits of the submerged mycelium.

The growth of *R. Crocorum* on malt-meat extract agar does not appear to "stale" the medium for the growth of *Penicillium* sp. as infections will grow actually on the old colony of the *Rhizoctonia.*

Malt and meat extract solution. Growth very good, commencing at once from the inoculum. It was noted that growth of a single colony per flask was no more rapid

than occurred from each colony when the inoculum broke up into many pieces and many colonies were formed. The mycelium appears colourless through the bottoms of the flasks although deep colour is developed in the medium. The first aerial hyphae to appear above the surface of the solution are colourless but after an "island" of growth has been built up the surface mycelium varies in colour from brown to purple. On filter paper supports growth is poor, but on wood it spreads until the whole block is covered with a purple-brown felt—compact above but showing considerable loose mycelial development near the surface of the liquid. Eventually small bodies resembling sclerotia (see p. 299) appear near the upper end of the wood.

Malt and meat extract gelatine acidified with citric acid. Growth good with development of an intense purple colour in the medium. At the top delicately violaceous close aerial mycelium is produced. The gelatine is slowly liquefied. In some tube cultures started during a spell of hot weather the inoculum became soon submerged owing to



Black = visible compact fungus tissue. White = agar, more or less coloured.

Fig. 1. Drawings to show the comparative amounts of growth over the surface of the colonies of *R. Crocorum* grown for five weeks at the temperatures indicated. The lengths of sections indicate the comparative diameters of the colonies. The black indicates the amount of visible very compact fungus tissue. At 19° C. the growth over the outer zone of the colony was too thin to be indicated exactly proportionally.

the melting of the gelatine and in these cases no colour developed until the mycelium grew up to the aerated zone. In a flask culture started on 23rd May, 1923, and kept in a rather cold laboratory the growth was as above at first—good, with the development of much colour and some purple aerial hyphae and slow liquefaction of the gelatine. After a week or two the whole of the gelatine melted during some unusually warm weather and then remained continuously liquid with the growth submerged. All the liquid steadily developed a very intense brownish purple colour. After some eight months from inoculation an "island" of growth 3–4 cm. in diameter appeared which was opaque white, but which gradually turned to a violaceous colour. Seen through the bottom of the flask the submerged mycelium appeared colourless.

Meat extract agar. Growth similar to, but not so abundant as, that on malt or on malt and meat extract agars. On thin layers of agar in plates which begin to dry out before growth has advanced very far a tendency to form the compact sclerotia-like

bodies mentioned above has been observed. The colour developed in the agar is more distinctly purple usually than when malt is present.

Jardox extract gelatine. Flask culture. Growth very slow, almost entirely submerged with only slight development of aerial mycelium. Gelatine is very slowly liquefied. After eight months at low laboratory temperatures the liquefied gelatine has a colour somewhat similar to that of a prune decoction. The mycelium appears colourless and the pigment developed in the gelatine has not extended into the non-liquefied portion.

Jardox extract broth. Very slow growth, the liquid turning deep brown.

Bouillon gelatine (not neutralised)[1]. Incubated at 19° C. there is no liquefaction of the medium in 3–4 weeks. At 22–23° C. there is slight liquefaction in the centre of the colony. Growth is fairly good but there is comparatively little pigment production. The main part of the colony is reddish brown in the medium with a practically colourless outer zone. Some practically white aerial mycelium is developed in the middle.

Prune agar. At laboratory temperatures growth is slow and of a dingy purple colour. Old cultures show a compact somewhat granular looking surface layer with practically no fluffy aerial hyphae. On freshly made agar incubated at 22–23° C. growth is much better and equal to that on malt agar. The mycelium is dark purple in colour, and the colony is surrounded by a deep claret-coloured zone of diffused pigment.

Soft acid cherry decoction agar. Fair growth. After seven months in flask culture a deep brown colour extended about 3 cm. deep into the agar. Some close "woolly" aerial mycelium, very light brown in colour, was developed, but over two-thirds of the surface it was so scanty that the dark colour of the agar showed through.

Quaker Oat agar. Growth good. Aerial hyphae short and tending to form minute light brownish clusters which were at first taken for an impurity.

Thin oat agar. Growth very slow. Eventually there is a fair amount of pale cinnamon-coloured aerial mycelium and a deep red-brown colour is developed in the medium.

Corn-meal agar. Growth exceedingly slow and scanty, scarcely visible after four weeks at 22–23° C. A deep violet colour diffuses from the inoculum into the fresh medium.

Potato agar. Very little superficial mycelium; moderate growth in the agar developing to a very deep brown colour. In plates incubated at 22–23° C. the colony shows at an early stage an irregular fan-like development.

Sterilised potato plugs. Growth very slow. The surface of the block becomes gradually covered with a deep brownish felt, with very little erect aerial mycelium. Eventually the mycelium extends into the cotton wool on which the block rests. Very old cultures show the potato completely penetrated by the mycelium and small aggregations of deep brownish mycelium here and there over the surface.

Steamed rice. Growth slow, with dull reddish mauve colour developed in the matrix.

Bread paste. Similar to growth on steamed rice, eventually the whole matrix being penetrated by mycelium.

[1] This was adapted from the medium used by Biourge (1923) for cultivating species of *Penicillium* and was prepared according to the formula: water 1000 c.c., Lemco 10 gm., peptone 10 gm., sodium chloride 5 gm., gelatine 120 gm., pure glycerine 24 gm., cane sugar 50 gm., cleared with white of egg.

Starch agar. Growth very slow with little superficial mycelium. The typical violet colour of the organism diffuses into the agar.

Dox's agar. Very slow and almost entirely submerged with only slight aerial growth: deep colour production in the agar. After ten months a considerable number of golden yellow crystals were found on the surface of the agar.

Fermi's solution and Uschinsky's solution. Practically no growth.

Milk. The inoculum sank and although there was a fair amount of growth after nine months comparatively little colour developed. The culture appeared almost white at the base with the remainder a dirty brown colour. The top $\frac{1}{4}$ inch remained liquid but below was an almost solid plug of growth. In a litmus milk tube growth occurred, by chance, at the surface and under the aerated conditions intense colour developed similar to that with the acidified malt and meat extract gelatine. The small amount of compact aerial mycelium was light violaceous in colour with some extruded drops.

Manure extract agar. Poor growth almost entirely submerged.

Peptone agar. (An acid medium developed by Waksman(10) for counting soil fungi.) Moderate growth in the agar with production of dark brown colour. In a freshly sterilised tube there was a fair amount of light brown aerial mycelium.

Clover leaf and stem extract. Very moderate growth.

Clover leaf and stem extract agar. Growth slow, not piling up above the surface at all. There is a slight development of aerial mycelium over the whole colony, giving a delicate violaceous tint viewed from above.

Sterilised clover stems. Growth very poor and soon ceased, probably owing to rather dry conditions.

Steamed oat straw and steamed wheat straw. Very slight growth.

Crocus corms. (Not *Crocus sativus*, but the ordinary English garden Crocus.) On corms which had been sterilised in mercuric chloride solution and washed with sterile water no growth at all occurred when inoculated and placed in moist chambers. This result may have been influenced by the degree of maturity of the corms, which were specially lifted early for use, or it may have been due to the species of Crocus used. On sterilised Crocus corms growth was slow and very light coloured at first but fairly abundant after ten weeks at 30° C., forming dark brown hummocks on the surface of the corms and appearing rusty brown in colour where it extended to the sides of the tubes. The brown colour extended throughout the moist cotton wool plugs at the base. Chopped up Crocus corms used for the preparation of a Crocus decoction were also sterilised in a flask and inoculated. On this rather poor nutrient material growth was very slow and chiefly of a rusty brown colour.

Crocus agar. Moderate growth, very deep brown with little aerial mycelium.

Thick Crocus decoction. Fair growth, liquid almost black, aerial mycelium rusty brown.

The optimum temperature for growth in culture.

When first isolated growth on all media and at the temperatures tried appeared to be extremely slow. At summer laboratory temperatures sufficient growth for making sub-cultures was obtained only after several weeks. It was desirable to obtain some rough idea as to the optimum

temperature for growth of the fungus, but before our experiments yielded reliable figures the slowly growing organism also served to emphasize the importance of the "internal condition" of fungi used in such physiological determinations. Owing to the slowness of growth of the *R. Crocorum* in our original transfers and the impatience of the authors the first attempts at obtaining some idea of the temperature relations of the fungus were made by inoculating poured plates of media with pieces of growth taken from a tube culture. The toughness of the growth and the paucity of the margin of advancing mycelium make it difficult to transfer any number of approximately equal sized pieces of mycelium or without varying quantities of the medium attached. The results obtained were most irregular, especially when a portion of agar not already thoroughly permeated by the mycelium was transferred. In that event frequently some considerable time elapsed before the fungus grew out from the inoculum on to the fresh medium—in one extreme case not doing so until after the lapse of 45 days.

It may be mentioned here that in the course of the work it has been noted also that the time that elapses before new growth starts in a sub-culture depends on the age of the culture from which the inoculum is taken. If a culture about six months old is used as parent it may be two or three weeks before any new growth can be detected. On the other hand, transfers from the growing margin of a comparatively recent culture (6–8 weeks) grow out almost at once, especially if a very small piece of inoculum is used and so placed that the mycelium is immediately in contact with the fresh food material.

Edgerton (12) in his work on the temperature relations of various strains of *Glomerella*—a comparatively rapidly growing fungus—recognised the error arising from differences in the "internal condition" of the portions of mycelium used as inocula. Using "a small bit of mycelium from the edge of a rapidly growing colony" he states that in taking his measurements "the growth of the first 24 hours was never considered." In our experiments with *R. Crocorum* the error due to this cause was so considerably magnified that, as mentioned above, using transfers of pieces of mycelium from a tube culture duplicate plates varied so much as to make the results quite worthless. Examples of such irregular duplicates—each pair being the diameters of colonies in millimetres after the lapse of the same interval of time and with other conditions equal, but the inocula being different portions from the same stock culture tube—are as follows: 26 and 53; 34 and 48; 9·5 and 28; 16·5 and 29. When, however, more elaborate technique was adopted perfectly con-

sistent results were obtained. The figures given in Table I illustrate the good agreement that was obtained on measuring the diameters of colonies on series of similar plates. Plate X further shows the regular nature of the growth in the colonies although by the time these photographs were taken (five weeks after inoculation) "staling" had set in and the margins of the colonies were becoming irregular.

Table I.

Diameters of colonies in millimetres 14 days after inoculation, on meat-malt extract agar.

(Each figure is the average of two measurements to the nearest ½ mm. in directions at right angles.)

Incubated at	19° C.	20	19·45	17	17	18·25	18·25	18·25	17·5	Av. 18·2
	25·5° C.	24·75	22	22·5	23·75	22·75	24·25	25·25		Av. 23·6
	30° C.	18·75	21	22·25	18·75	20·75	20·75	21	20·5	Av. 20·5

Meat-malt extract agar was used as this was found to give the best growth of any of the media tried. In order to obtain a moderately deep layer of agar 30 c.c. were used to a petri dish 4 in. in diameter—the dishes being a good flat-bottomed sample. The method of inoculation was that used by Fawcett(11), equal sized slabs 2·5 × 3 mm. in diameter being cut with a Keitt platinum cutter round the edge of a regularly growing colony in a petri dish and one of these slabs being inverted on the middle of each fresh plate of medium. The fungus grew out on to the fresh agar in all cases practically at once and it was not necessary to discard any of the plates as possibly giving erroneous measurements. Table II shows the average increases in the diameters of colonies for successive weekly periods with one set of plates incubated at the temperatures given. Each colony was measured in two directions at right angles to the nearest ½ mm. and each figure is the average for seven or eight plates at each temperature. The laboratory temperature to which the one group of plates was submitted was rather low and variable, ranging from 7° to 15·5° C. and averaging about 11° C. over the 24 hour day and night period. A set of colonies which had been grown at a temperature of 30° C. for 3–4 weeks was transferred to an incubator at 37–40° C. No growth at all took place at this temperature and the young hyphae appeared to be killed by the exposure as on transferring to 25° C. no growth took place at the edges of the colonies. After 10 days at 25° C., however, a striking growth of almost colourless hyphae was taking place from the middle zones of practically all the colonies. This greater resistance of the median zones of the colonies seems to agree

with the results of attempts at isolation—the more resistant and viable portions of the fungus being found to be the sclerotium-like bodies.

Fig. 2 shows graphically the relation between the diameters of the colonies and the temperatures at which they were grown at times three and four weeks after inoculation of the plates.

A second set of plates was started on meat-malt extract agar on a batch of medium which had been prepared at a different time from that used for the above and the inocula were from a different secondary stock culture plate. There were nine or ten plates in the group for each temperature and the results obtained were exactly similar to those given in Table II for the first set. The average figures for this second set are shown graphically in Fig. 3. It is regretted that with the limited amount of apparatus at the disposal of the authors it has not been possible to obtain the growth figures at definite 10° C. intervals so that no temperature coefficients can be calculated.

It will be seen from the results that at all temperatures, except the low variable laboratory temperature, the rate of growth increased with each seven-day period until the end of the third week from inoculation. The rate of growth then steadily decreased, the decrease being most rapid and the "staling" most marked at 25·5° C.

Table II.

Average increases in diameter in millimetres for successive seven-day periods of colonies of Rhizoctonia Crocorum *grown at the temperatures indicated on meat-malt extract agar in moderately deep equal layers in petri dishes.*

(Each figure is an average from seven or eight dishes and the diameter of each colony was measured in two directions at right angles to the nearest ½ mm. $2\frac{1}{2} \times 3$ mm. was deducted for the size of the slab of the original inoculum.)

Temperature	1st period	2nd period	3rd period	4th period	5th period	6th period	Average total increases in diameter of colonies from time of inoculation (see Fig. 2) In 3 weeks	In 4 weeks
7–15·5° C. Av. 11° C.			5·9*	5·8	8·1	8·5	5·9	11·7
19° C.	3·6	11·8	13·6	12·8	11·65	10·45	29·0	41·8
25·5° C.	5·1	15·7	16·9	15·1	9·4	7·35	37·7	52·8
30° C.	4·9	12·8	14·4	12·3	10·75	lost	32·1	44·4
37–40° C.	Nil, after developing for 3–4 weeks at 30° C.						—	—

The results of a second similar set are shown graphically in Fig. 3.

* Total of first three periods.

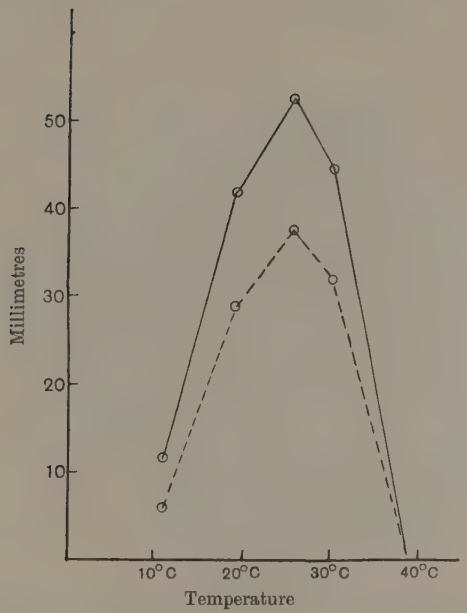


Fig. 2. Graph showing the comparative increase in diameter of colonies of *Rhizoctonia Crocorum* at different temperatures on meat-malt extract agar in three weeks – – – – – – and in four weeks ———— from inoculation. (The temperatures for the values indicated by points at 11° C. and 39° C. are approximate only.)

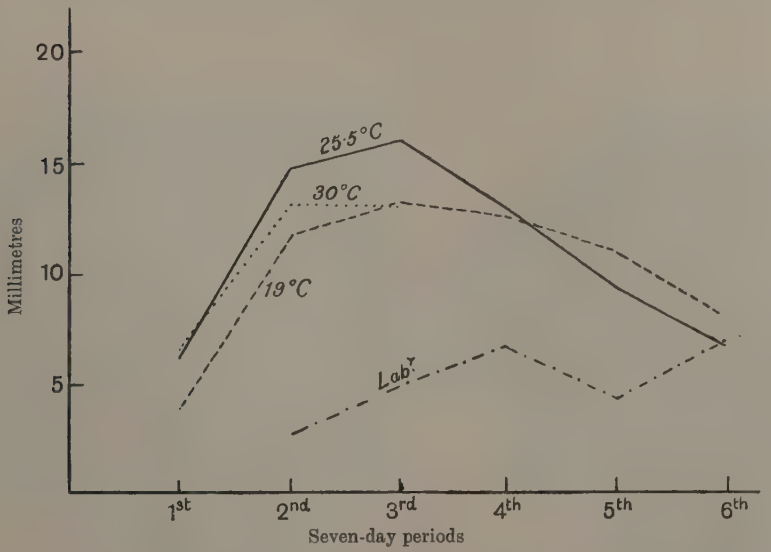


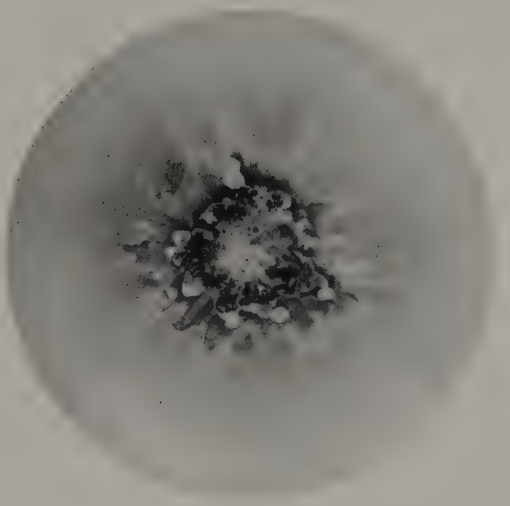
Fig. 3. Graph showing the increase in diameter of colonies of *Rhizoctonia Crocorum* for consecutive seven-day periods from the time of inoculation at the temperatures given. (The readings for 30° C. after three weeks were not obtained in this set.)

Low lab. temp. (av. 11° C.) – • – • – • – • – • 19° C. – – – – – – – – – – – – – –
25·5° C. ———————— 30° C. . . . . .

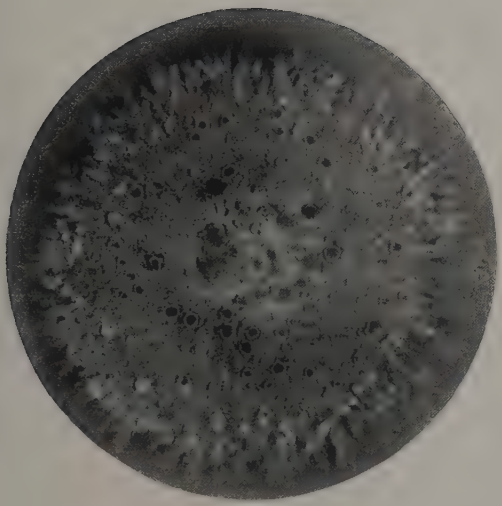
Working with his culture contaminated by a *Fusarium* Diehl states: "There was no apparent relation of various temperatures to this culture as exhibited in forty days growth on 'radicicola' agar, Thaxter's potato hard agar, and alfalfa root plugs at 10°, 20°, 22° to 25°, 30° and 37° C." On the other hand, Faris, working with potato plants grown at 90–100° F., obtained good growth of the *R. Crocorum* from infected "sets" whereas plants grown at 60–70° F. did not develop the disease. He states: "there is some indication that the fungus develops best at high temperatures." Our results given above show definitely that the optimum temperature for growth of *R. Crocorum* in culture, as measured by the increase in the diameter of colonies on meat-malt extract agar plates, lies in the neighbourhood of 25·5° C.—possibly a degree or so higher if, as is likely, the fungus shows a smooth temperature-growth curve similar to that given by other organisms of which more complete records can be found in the literature (cf. Fawcett).

Previous writers have attempted to correlate the occurrence of the disease in the field with the probable moisture relations of the fungus. Thus, according to Duggar, du Hamel represented the violet root fungus as selecting dry localities; the Tulasne brothers reported that while wet weather may give the fungus an advantage, it is found in the driest situations permitting crop growth; the fungus occurs very generally in southern Europe, especially in southern France and Italy, and in the United States it has been reported largely from regions of lower humidity and lesser rainfall. The temperature of 25·5° C., which has been shown to be the optimum for active growth of the organism, is high for a field parasite in this country, and, indeed, it is rare to find more than a few plants attacked in very limited areas in the field here. While, no doubt, moisture plays an important part in affecting the activities of the parasite, the authors are of the opinion that temperature is probably the most important factor in governing the relative abundance of the fungus in the various countries from which it has been reported.

The more definite knowledge concerning the temperature relations of *R. Crocorum* which has now been obtained is of importance in that it will allow of a more satisfactory interpretation of the results of cross-inoculation experiments and a sounder determination of the host range of the organism.



(*a*)



(*b*)

(*c*)

BUDDIN & WAKEFIELD.—Some Observations on the Growth of *Rhizoctonia Crocorum* (Pers.) DC. in Pure Culture. (pp. 292—309.)

SUMMARY.

The organism dealt with in this paper is that which has been commonly known in this country as *Rhizoctonia violacea* Tul. but until satisfactory proof is forthcoming of a spore stage of the organism it should be known by its valid prior name, *Rhizoctonia Crocorum* (Pers.) DC.

The methods used for isolation of the fungus in pure culture are described and some account given of preliminary inoculation experiments with the re-isolation of the parasite.

It is shown that growth of *R. Crocorum* will occur on a very wide range of sterilised artificial material when in pure culture.

No spore form has occurred in any of the large number of cultures which have been grown.

Some notes are given on difficulties arising in temperature relation work from differences in the "internal condition" of the organism under varying conditions, and it is shown that the optimum temperature for the growth of the organism in culture on meat-malt extract agar as measured by the increase in diameter of colonies is in the neighbourhood of 25·5° C. This optimum temperature probably explains the geographical distribution of the severe attacks of the organism in the field.

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EXPLANATION OF PLATE X.

Photographs showing the appearance of colonies of *Rhizoctonia Crocorum* after growing for five weeks at (*a*) 19° C., (*b*) 25·5° C., and (*c*) 30° C. (Nat. size.)

Photos by G. ATKINSON.

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